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Potential Analyzers of Toxic Materials for On-Line Use

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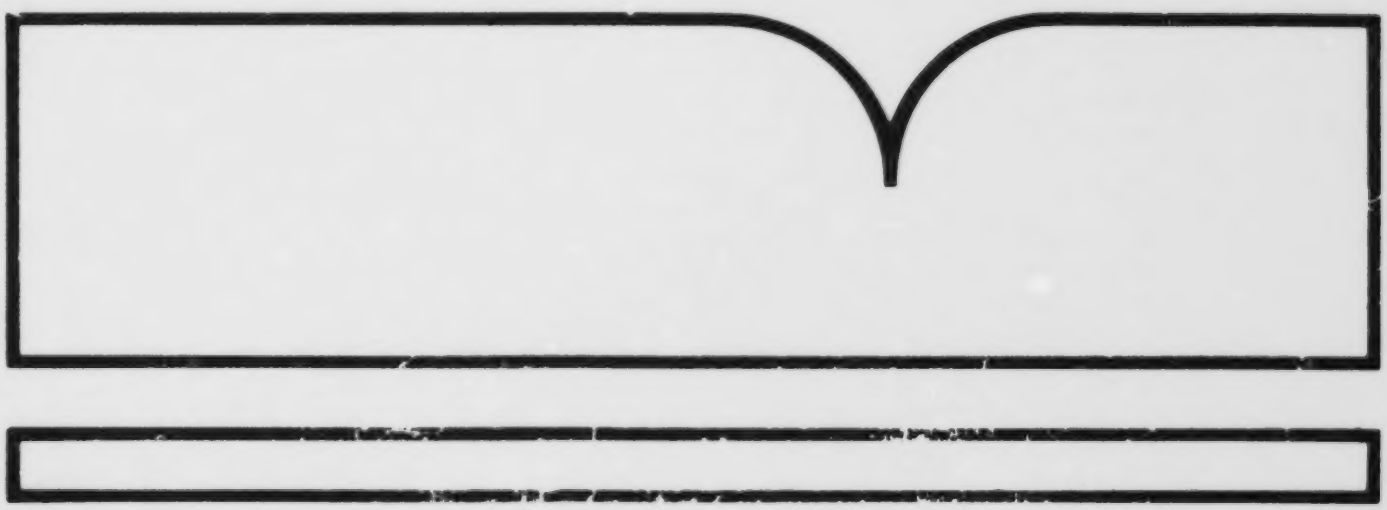

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POTENTIAL ANALYZERS OF TOXIC MATERIALS FOR ON-LINE USE

by

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16. ABSTRACT

There is no universal toxic monitor capable of monitoring all materials that are toxic to humans. The best that can be done is to qualitatively detect the presence of the most likely toxic materials in certain applications. In this paper, five classes of analyzers are reviewed that are capable of detecting one or more toxic materials or their surrogates. Recommendations are made to automate each of these systems for on-line operations. The classes of analyzers as used in this paper are: life function monitors, enzymatic response, respirometers, organic halide monitors and specific monitoring for surrogates using such equipment as on-line gas chromatographs.

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INTRODUCTION

The purpose of this paper is to search the literature and document biological and chemical methods which are amenable for use in the development of an on-line toxic monitor for drinking and recreational waters. The advantage of an on-line monitor is that real-time or near real-time data is available. As the concern for toxic analysis grows there will be increased emphasis on real-time monitoring to provide an early warning signal of the presence of toxics.

During the past 20-30 years, there has been a search for a single, all-purpose monitor or monitoring method to determine the effect of adverse environmental conditions[1] on the state of human health. This search for a toxic monitor is complicated by the fact that toxic materials appear to affect each species of animal differently. According to Cairns and Van Der Schalie,[1] water quality (i.e., hardness, dissolved oxygen concentration, pH, temperature, etc.) has a very marked influence on the expression of toxicity. Consequently, before useful management information on potential toxicants can be obtained from an on-line toxic monitor, considerable lab testing must be done.

It is difficult for the scientific community to agree on a standard for on-line toxic monitors, even though many methods are ingenious and do give a good early warning alarm in the presence of toxics. An automatic method may disagree with some other index or reference for determining toxic material, adding to the complexity of the problem. All this leads one to the conclusion that there is no universal toxic monitor capable of monitoring all materials that are toxic to humans. It would appear, therefore, that on-line analyzers of toxic material will be able to serve only as early warning devices to alert the lab for further testing.

DISCUSSION

In order to address the subject of on-line toxic analyzers, the potential analyzers may be classified into five different categories. Each category of analyzer has the capability of detecting one or more toxic materials or their surrogates. In this context, a surrogate may or may not be a toxic material, but it is easily identified and is usually found in the presence of toxics. The categories of analyzers are: life function monitors; enzymatic response; respirometers; organic halide monitors; and specific monitoring for surrogates using such equipment as on-line gas chromatographs. Respirometry is considered as a separate category because of the extensive work that has been done in this area and also the number of such on-line instruments that are available.

Life Function Monitors

A life function monitor may be defined as a device using some life form,

e.g., fish, microorganisms, plants, or animals to monitor toxic environments. As the toxic materials increase in the environment, the organisms react to the situation by the death, decreased, or increased activity. This entire process is designed and selected in such a way that any disturbance of the species can be quantitatively measured.

Buikema, et al.[2] estimated that there are over 165 test organisms that have been proposed and of these, 65 are macroinvertebrates that fulfill the qualifications necessary for toxic testing. Each one of these potential life function monitors must meet the rigid conditions necessary for toxic monitoring as outlined by Cairns and Van Der Schalie[1]. These conditions are:

1. The physiological or behavioral parameter of the organism should be quantifiable through the use of a computer or other electronic recording equipment.

2. The organisms should exhibit rapid, reliable detection of developing toxic waste conditions.

3. The monitoring system should have a minimum of false responses to nontoxic situations.

4. The system should lend itself to established methods for data handling and analysis.

5. The monitoring systems should be relatively easy to operate and should produce results relatively easy to interpret.

6. The selected organism should be fairly inexpensive and relatively easy to acquire.

7. The monitoring apparatus should be reliable and require little maintenance.

Using the above conditions as a guide, a large number of unique methods have been developed and automated, but some of the methods are very complex and require extensive plumbing systems. A review of these methods is presented by Cairns and Van Der Schalie[1] and some of the automated methods are highlighted below.

Experimental procedures that have been used in monitoring toxic chemicals involve such methods as cough reflex, oxygen consumption, movement patterns, avoidance and rheotaxis of aquatic animals. Peltier[5] discusses some of the methods and describes the apparatus and theory. He has revised and updated a manual for acute toxicity testing and it is now in the process of being printed.

Many of these methods require large automated test chambers, complex plumbing and frequent manual checks with respect to the health and welfare of the test animals. An abridged listing of some of these systems is given below to show the reader the types of systems that have been used. None of these systems will be considered for an on-line field monitor because too

much manual effort is required to assure the health and welfare of the test animals.

Kapoor[6] placed aquatic insects on a small beam which was connected to strain gauges. Any respiratory body movement of the animal deflected the small beam which imposed a stress on the strain gauge and produced a proportional electrical signal.

Huesner and Enright[7] measured the activity of the test animal (fish) indirectly by sensing the heat loss from a thermister caused by the water currents produced by the animal's movements.

Maciorowski et al.[8] monitored the movements of several crayfish in individual chambers. The movements of the crayfish (apparently muscle contraction) created small electrical signals, and the resulting information was processed by a microcomputer.

An automatic monitoring system based on the breaking the light beam of three red light photocells by a single fish to record the total activity of bluegills has been developed by Waller and Cairns.[9] This system was later modified to feed the data into a minicomputer.[10]

Rheotaxis, which is the ability of a fish to maintain its position in a current, was used by Poels[11] in an automatic system employing photocells. Lab-scale experiments showed that acute toxic levels can be detected before death occurs, but when the system was tested in Rhine River water for 18 months, no toxic alarms occurred.

Kurt[12] also used rheotaxis in his patented system. He placed the fish in a chamber with wire meshwork at each end so that each time a meshwork was touched by the fish, a signal was emitted. When water was allowed to flow through the chamber, the fish would orient themselves against the flow. However, if toxics were present, the fish would orient themselves with the flow. The frequency of signals from the upstream and downstream meshes was interpreted as an early warning signal of the presence of toxic materials.

On-line analyzers which would be required to operate for long periods of time unattended would require a different approach than those listed above where the health and welfare of the test animal must be routinely monitored. Recognizing the emphasis today on selective electrodes Karube et al.[13] studied microorganisms as part of two microbial electrodes using a recombinant deficient (Rec-) strain, Bacillus Subtillis (Rec-) and a wild strain, B. Subtillis (Rec+) to screen mutagens. The principle of the sensor is based on the inhibitory action of the mutagens or the respiration by the B. Subtillis (Rec-). In the presence of chemical mutagens, the current of the Rec-electrode gradually increased while the Rec+ electrode current remained constant. A linear relationship was obtained between the rate of current increase of the Rec-electrode and the mutagen concentration. The electrodes were also found to be sensitive to antibiotics, respiration inhibitors and bactericides. Figure 1 is a schematic diagram of the electrode system.

Using microorganisms as sensors, an ambitious automatic on-line toxic monitor was developed and studied by Jeffers and Taylor.[3,4] The system consists of three bacterial biomass monitoring devices. In the first

device, total bacteria counts are measured by light from a chemiluminescent reaction catalyzed by the porphyrins from lysed bacterial cells. If carbon monoxide is added to the reaction, then it becomes possible to obtain the viable cell count. In the second device, an estimate of living bacterial biomass is made by assaying adenosine triphosphate, ATP (using the luciferin-luciferase reaction). In the third device, total coliform and fecal coliform levels are estimated by measuring the hydrogen gas evolved by these organisms in the presence of lactose. In each case, the presence of toxics would influence the viable bacteria count and affect the production of light or hydrogen gas. The complete system designed for intensive water quality monitoring is automated by two computers and housed in a mobile trailer.

In the United States, a manually operated toxic analyzer in which microorganisms are handled as chemical reagents has become commercially available . The Beckman Microtox™* utilizes a specialized strain of luminescent bacteria which when exposed to toxic materials emits a quantity of light inversely proportional to the toxicant. When several dilutions of the toxic material are made and analyzed, an EC 50 (the concentration which causes a 50% reduction in the light produced over a specified period of time, e.g. 5 minutes) can be obtained.[14] Figure 2 is a schematic illustration of the Microtox.

*Mention of trade names or commercial products in this paper does not constitute an endorsement or recommendation for use by the U. S. Environmental Protection Agency.

There have been several papers published on the use of this instrument, and Beckman Instruments also did considerable research on the performance of the Microtox system. Their results are summarized in the four papers by Bulich et al.[14,15,16,17]. By exposing the bacteria to the toxicants for 5-minute intervals, Bulich found EC 50's of 0.065, 0.5, 0.7, and 1.5 mg/L for mercury II, pentachlorophenate, aroclor 1242 (PCB) and p-cresol, respectively. He obtained good correlations when he compared the pure compound Microtox data to 96-hour fish assay using the Lethal Concentration (LC-50) data that he obtained from the literature. He also compared the 5-minute EC 50 data for the Microtox with 24-hour LC 50 fish assay data while testing 20 different industrial and wastewater effluents and noted good comparisons. A number of other investigators evaluated the Microtox; and in general, their results were favorable. For example, Samak et al.[18] also found good agreement with the Microtox and bioassays using zebra fish.

Chang[19] investigated the response of the Microtox to certain pure chemicals such as ethanol, benzene, phenol, etc. and compared the results with information on the toxicity of those chemicals to rats (oral ingestion), Lethal Dose (LD) 50 (mg/kg) and fish LC 50 (mg/L). He calculated a correlation coefficient of 0.90 for the Microtox with the rat toxicity tests and 1.0 with the fish toxicity assays. However, when he compared the toxicity of selected pesticides (e.g. captafol, carbaryl, DDT, Malathion, etc.) as measured by the Microtox to the rat toxicity tests, he found a much lower correlation coefficient of 0.09.

Dutka et al.[20] observed great variations in the sensitivity patterns of four microbial toxicity screening procedures to selected chemicals. Some of these chemicals had toxicity end point concentrations which were 100-1000

times greater from one test system to the next; e.g., the EC 50 for Zn was 3.5 ppm in the Microtox system and 370 ppm using the Pseudomonas fluorescens test. Another example is the EC-50 for sodium lauryl sulfate which was 1.8 ppm for the Microtox system as compared to 3700 ppm for the Aeromonas hydrophita test. With the exception of Hg, Ni, Pb and N-nitrosodiethylamine, they concluded that the 15-minute Microtox test was the most sensitive to the chemicals tested.

An extensive study involving 99 samples of wastewater was conducted by Peltier and Weber[21] who compared the response of the Microtox to acute fish and invertebrate bioassay tests. Their unpublished work showed that of the 43 samples that were toxic to fish, 35 (81%) were found to be also toxic as measured by the Microtox test. Of 99 wastewaters originally tested, 16 samples were not toxic to fish but were toxic according to the Microtox test. Finally, there were 40 samples of the 99 that were not considered toxic to either fish or Microtox test. These data are illustrated in Table 1.

Table 1
Comparison of the Toxicity of Wastewater Samples

	Fish Assay Comparison		Invertebrates Comparison	
	Fish	Microtox	Invertebrates	Microtox
Total Number of Wastewater Samples	99	99	54	54
No. of Toxic Samples	43	35	42	25
Samples Toxic Only to Microtox		16		3
Samples Not Toxic to Either Test	40		9	

When compared to invertebrates, of the 42 wastewater samples that were toxic to the invertebrates, only 25 (60%) were also toxic to the Microtox system. There were also three samples of the 54 wastewater samples originally tested that were not toxic to invertebrates but were toxic to Microtox, and nine samples that were not toxic to either test system (see Table 1).

Beckman now recommends a 15-minute exposure time (the time the toxic material is exposed to the lumeniscent bacteria) at a constant temperature of 15°C in order to improve the sensitivity of the analyzer to toxics.

The Microtox system should be explored further for on-line automation as proposed in Figure 3. The toxic sample is collected, mixed with luminescent bacteria and diluent (to provide osmotic protection for the luminescent bacteria) and pumped into vial A. Vial B is filled with distilled water, diluent and luminescent bacteria. The amount of distilled toxicant or water that is added is the same for vial A and B, and the light output from both vials is monitored. The materials are pumped into the vials using a plunger mounted on "O" rings which, in addition to moving the solutions, also cleans the walls of the vial. Distilled water will be used as a rinse to assure the availability of clean vials for test samples.

Enzymatic Response

Another approach to the measurement of toxics is the use of enzymes or metabolites which generate a measurable response in the presence of toxic materials. Adenosine triphosphate (ATP) has been studied as such a metabolite, and has been detected by the light emitted from the luciferin-luciferase enzyme reaction. Toxic materials adversely influence the response and cause a decrease in the amount of light emitted.

The method has been found to be rapid and sensitive enough for routine use, Kennicult[22]. He found that chemicals such as chloroform, mercuric chloride, and acrolein when added to a standard solution of ATP decreased the emitted light indicating significant decreases in luciferase activity. But he also noted that some chemicals, e.g. acetone, which appears to act as a carbon source, caused an apparent increase in ATP concentration because the emitted light increased.

Turner Instruments[23] prepared a list of common sources of error in the use of the luciferin-luciferase reagent and those which may interfere with an on-line monitor are mentioned below. They concluded that clean, easily wetted glass can inactivate the reagent and recommended the use of non-wetting polypropylene or similar materials. They also observed that

dissolved salts inhibit the reaction and cited as an example that the use of sea water with 3.5% dissolved solids reduces luminescence by a factor of 20.

Since it appears that ATP content may increase or decrease depending on the chemicals or microorganisms present, ATP may not be a suitable parameter for measuring toxics.

A successful enzyme monitor that was developed and demonstrated by the U. S. Environmental Protection Agency (USEPA), employed the enzyme cholinesterase.[24] The Cholinesterase Antagonist Monitor (CAM-1) uses a pad of immobilized cholinesterase (ChE) which is sensitive to nerve agents and organophosphate and carbamate[25] pesticides. The water sample is pumped through the enzyme pad for a period of two minutes.[26] During the third minute of the three-minute cycle, air is blown through the pad to displace the residual water. Then, a solution of substrate consisting of butyryl thiocholine iodide in tris (hydroxymethyl aminomethane) buffer is pumped through the enzyme pad. In the absence of enzyme inhibitors (in this case organophosphate and carbamate pesticides), the cholinesterase hydrolyzes the substrate to give thiocholine iodide. The chemical equation for its reaction is:

$$C_3H_7COSCH_2CH_2N^+(CH_3)_3\ I^- \xrightarrow{ChE} C_3H_7COOH + HSCH_2CH_2N^+(CH_3)_3I^-$$

The resulting thiol, a mercaptan, is easily oxidized at low voltages in a constant current electrochemical cell resulting in a disulfide bond,

$$HSCH_2CH_2N^+(CH_3)_3I^- \xrightarrow{Oxidation} (CH_3)N^+-CH_2-CH_2-S-S-CH_2-CH_2N^+(CH_3)_32I^-$$

On the other hand, in the presence of enzyme inhibitors or the absence of enzyme, the substrate is not hydrolyzed, and since the original substrate butyrylthiocholine iodide is not readily oxidized, the voltage in the electrochemical cell rises to about 200 to 400 mV.

For the purposes of this paper, the description of this mechanism will be assumed as the explanation for operation of the analyzer. In fact, the exact mechanism is not known, and there are still several unanswered questions about the mechanism. For example, it appears that the electrodes are gradually conditioned to the reaction, resulting in a drift of electronic potentials. Although the mechanism for the cause of the drift is not understood, the problem has been solved using the electronics of the CAM-1 analyzer.[26]

The instrument is capable of detecting such pesticides as dimethyl, 2,2-dichlorovinyl phosphate (DDVP) at 1.0 ppm at 5°C or at 0.2 ppm at 25°C. Sensitivities for other organo phosphorus and carbamate pesticides showed similar changes with temperature. Of 29 pesticides studied, sensitivities varied from 0.1 ppm to 70 ppm. The analyzer is less sensitive to the phosphorothioates and dithioates and thiocarbamates. The reason for the enzyme inhibition on loss of sensitivity for these sulfur containing compounds is not understood.

Two reports[27,28] list in excess of 50 other enzymes which are ammenable to the CAM-1 system, although only limited experimental data is

given. Currently, USEPA is evaluating the enzyme lactate dehydrogenase (LDH) for use as a quick, simple (CAM-1) detector for chlorinated hydrocarbons that are equal or larger in size than chlorinated benzenes. The inhibition by chlorinated hydrocarbons of the LDH catalyzed reaction can be monitored colorimetrically or by pH. Sensitivities to 10^{-5} M concentrations of Aldrin, toxaphene, DDT, Aroclor 1242, and pentachlorocaphenol have been demonstrated in the laboratory.[29] The cholinesterase technique was described in this paper because it does work and appears to have potential for application to monitoring other toxics. However, the use of CAM-1 for monitoring general toxics in water is limited. LDH appears to have potential because it is focusing on monitoring organic halides which could be used as indicators of other toxics, but considerable research remains to be done on the LDH approach before it can become a field instrument.

Respirometers

Since respirometry measures the oxygen utilization of living animals, it serves as a good technique to monitor their metabolic activity. The size of the organisms utilized in the respiration measurements have varied from fish to small microorganisms. Regardless of the size, the presence of toxic materials will disrupt the normal metabolism and, as a consequence, hinder respiration or kill the animal.

Toxic materials do not always yield such pronounced respiratory effects as cessation of respiration on larger animals. For example, in small

concentrations, toxic materials may affect the ventilatory (opercular) movements of fish. The fish may respond by attempting to reverse the flow of water in the gill and discharge the toxic material. This action is sometimes referred to as the "fish cough." These procedures have been studied extensively, and the fish cough test is amenable to automation. A review on the aspects of monitoring fish coughs was authored by Drummond and Carlson.[30] In a more recent report by Gruber et al.[31], solid wire electrodes were fastened at the ends of a fish tank to monitor the disturbance of electrical fields about a fish caused by its ventilatory movements. The data collection system was complex and utilized a minicomputer.

The more classic approach to the respiration measurements would involve sealing the animal in a closed container and monitoring the oxygen level or pressure changes. Cairns and Van Der Schalie[1] have written a detailed review on the subject of using aquatic animals in classical respiration measurements to detect toxics. They noted that the difficulties in making these measurements in the field are the lack of control of such variables as temperature,[32] oxygen level,[33] day length, reproductive development,[34] and the activity level[35] of the aquatic animals during the tests.

In spite of these disadvantages, both Callahan[36] and Tackett, et al.[37] developed devices which automatically measure the respiration rate of the fish in a chamber which was automatically flushed with water and then closed for monitoring. Callahan monitored toxics in wastewater while Tackett, et al. worked with river water. Using an electrolysis cell, the oxygen was maintained at a fixed level during the measurement cycle. The amount of oxygen generated was proportional to the amount of electric

current used, which is a function of the respiration rate. All values were recorded automatically.

In a related device, Kitsutaka[38] measured the difference between the oxygen content of wastewater entering and leaving a fish chamber. If too small a difference were noted, an alarm was sounded to indicate the death of the fish and the possible presence of toxic materials.

For this application, microorganisms, because of their large numbers in a small volume and their rapid reproductive rates, may be less sensitive to the problems plaguing larger animals. If respirometry is to take advantage of this approach then a reliable reservoir of healthy microorganisms must be maintained. Using these thoughts, Slabbert and Morgan[39] have found that the protozoan Tetrahymena pyriformis provides an excellent respiratory response to toxic materials when used to monitor water quality. They claim that T. pyriformis has a fast response and is easily reproduced and maintained in the laboratory.

They also describe a device utilizing a dissolved oxygen (DO) probe to monitor the respiration rate in a specially designed test chamber. The addition of toxicants caused a change in the rate in which DO is being depleted from the chamber. They studied the effects of nine toxic materials with their device. Without further modification, the organism and the associated respiratory technique did not appear to have sufficient

sensitivity to detect pollutants at the maximum allowable concentration level for drinking water.[39]

There are two concerns in using respirometric measurements: 1) are there sufficient microorganisms to cause a response, and 2) is the response of the organism sufficient to detect toxics. In the example above, an organism more sensitive than T. pyriformis may be necessary to monitor toxics in drinking water. In the example below, other sources of microorganisms are explored.

Larson and Perry[40] observed that the microorganisms in a river one mile downstream from the effluent of a municipal wastewater treatment plant were sufficient in number to provide a seed for monitoring toxics using respirometry. They described a manually operated electrolytic respirometer which was used to detect toxic chemicals. In a later paper, Larson and Schaeffer[41] found that the mixed liquor from an activated sludge treatment plant was an excellent source of microorganisms for toxicity studies using the same respirometer as Larson and Perry.

A very complex automatic respirometer called the Toximeter was developed and evaluated by Pagga and Gunthner.[42] The Toximeter is actually a pilot-scale wastewater treatment unit which measures semicontinuously the respiration activity of the microorganisms and sounds an alarm if the respiration decreases significantly. The device includes a primary and secondary clarifier with provision for returning the sludge to the measuring and controlling unit (aerator). Such parameters as DO, turbidity, pH,

temperature, and TOC are continuously and automatically measured on-line. Only DO is used in respirometer measurements to detect toxic chemicals. The remaining parameters collect information to ensure the efficient operation of the Toximeter.

A less complicated respirometer called Toxigard which may after further research have application as an on-line toxic monitor was developed in Sweden.[43,44] The device automatically feeds synthetic sewage or a mixture of synthetic sewage and industrial effluent into an aerator for a fixed period of time and then into a biological filter. The DO of the effluent is measured after the mixture has had time to mature at which point a determination is made whether the mixture is toxic. The authors cite several case histories in which the Toxigard has proven successful in timely detection of toxic chemicals.

Arthur[45,46] outlines the general theory of using respirometry to determine toxicity and provides some historical insight into respirometric measurements. He recommends that the organisms be brought to the endogenous state before the unknown sample is introduced. If the unknown sample has food value and is nontoxic, the oxygen uptake increases above the baseline of endogenous respiration. On the other hand, if the unknown sample is toxic, little if any increase in the baseline occurs. He also describes an on-line instrument which is submerged in a water jacket in which the temperature is maintained closely to the temperature of the source of the sample, thus providing the advantage of monitoring the respiration rates at the sample temperature and not at the lab temperature. Other obvious

advantages are its ability to produce continuous and timely data without excessive labor costs. He recommends that if advance warning is of primary concern, a location upstream be selected with provision for a seed tank to be used to mix small portions of return sludge and process wastewater prior to introduction into the respirometer.

By comparing changes in respiration with the baseline respiration or the endogenous rate of respiration, Arthur noted that sometimes it is not possible to differentiate between a mildly toxic sample and one not easily treated. The respirometric response in both cases is similar, and either condition could result in insufficient metabolism and a high BOD_5 in the final effluent. If such conditions are suspected, then he recommends further tests using a laboratory respirometer. Another problem he noted was that low initial rates of respiration may actually be a time of the microorganism's acclimation to the toxic material, and the initial lag in response would be followed by maximum rates of respiration as acclimation to the waste takes place. This, too, can be easily determined by performing additional tests with a laboratory respirometer.

The technique of measuring the respiration rate of microorganisms to detect toxics shows promise because on-line equipment is commercially available and the microorganisms can generally be managed easily. Due to the large numbers of microorganisms that can be utilized in a small container and their resilience as contrasted to fish, such concerns as the general state of health of the animal and feeding times are minimal. Nevertheless, considerable research is needed before respirometry can be

used to monitor water quality. Such research should include the development of sensitive microorganisms which can be used as seed, an investigation of the sensitivity of the technique and, if necessary, mechanical alteration of existing equipment so that greater sensitivity is obtained. And finally, the equipment should be evaluated in the field for monitoring water quality.

Organic Halide Monitors

Another approach to the detection of toxics is the chemical analysis of surrogates. Surrogates, by their definition, are limiting, and considerable thought must be given to the proper selection of not only the surrogate but also to the sample location and monitoring objective. If the objective is to protect the general public from possible toxicants entering the water supply, a suitable surrogate may be total organic halides (TOX). It has been shown that drinking water treatment processes in which effluents are chlorinated may also generate TOX which will eventually enter the water supply.[47] The detection of TOX, therefore, not only indicates the presence of organic halides, but may also reveal the presence of other non-halogenated toxic organics in the water supply. In other words, TOX could serve as an alarm for toxic chemicals. Consequently, it would be desirable to develop a TOX analyzer to monitor drinking water, and in the event that TOX is present above an arbitrary limit, a more detailed analysis should be undertaken.

Today, the only analyzers which claim to have the capability to analyze TOX are manually operated. The principle of operation consists of

combusting the organic halide and converting it to a halogen acid (HX) whereupon it is measured either by electrochemical methods or by conductivity. The analyzers have the capability of determining both the purgeable and nonpurgeable organic halides by adsorption and desorption on activated carbon or by its use of the purge and trap method of Bellar and Lichtenberg.[48] It appears that both devices are amenable to automation by modifying carrier gas routes and controlling the operation with a timer.

A unique approach to the measurement of TOX was presented by Driscoll and Hewitt.[49] They coupled a photoionization detector (PID) and a flame ionization detector (FID) in series and compared the ratios of PID/FID. It appeared that if the ratios were high the chances were better for the presence of chlorinated hydrocarbons. Another related technique they have suggested is the use of an electron capture detector (ECD) together with a PID to obtain a PID/ECD ratio. This system may prove more sensitive to chlorinated hydrocarbons.

The PID used in the above experiments had a lamp energy 10.2 eV. Use of the PID with 11.7 eV would detect almost all materials, while the 9.7 eV lamp would react to materials such as acetone but not hexane. This approach has considerable flexibility and potential, but the technique has not yet been fully explored as a general warning device.

Gas Chromatography

The application of automatic on-line gas chromatography is, as a rule,

limited to monitoring only organic effluents from industries. This is because the analyzer must be set up for a particular class of chemicals to accommodate a single column chromatograph and then, using automatic procedures, is limited to analyze only the number of chemicals per sample for which it has data ports. This limitation of the number of data output ports and the fact that different classes of chemicals require different gas chromatographic columns limits the application of the analyzer to effluents of predictable composition in which only spills are being monitored. Nevertheless, the technique can monitor surrogates from a particular industry or an entire class of similar industries in a water basin area. Furthermore, there are several commercial units available on the market which have the capability to use not only FID, but also ECD and PID detectors. In addition, some of these units are capable of temperature programming, and others even have two or more columns for increased flexibility.

The use of automatic gas chromatography was expanded by Kerner, et al.[50] who explored the concept of extraction followed by a gas chromatograph analyses for organic halides. They outlined the procedure for automatic extraction of the sample followed by on-line gas chromatographic analyses using an ECD. The system included automatic internal calibration at selected times and showed recoveries of 87-98% chlorinated pesticides if the ratio of the volume of solvent (hexane) to the collection time is low 2-0.25 L/hr, respectively.

Low molecular weight volatile organics were successfully analyzed automatically by a computer-based gas chromatograph.[51] The volatile organics are thermally desorbed from the water sample and displaced into the headspace by purging the sample with ultrapure helium. The displaced organics are then adsorbed on a Tenax column which fits into the injection port of a modified gas chromatograph. On-line data collection and reduction are achieved by a microprocessor. Automation of the procedure results in improved data reproducibility and allows monitoring of industrial and wastewater treatment plant effluents.

Conclusion

Only a few techniques exist for on-line monitoring of toxic chemicals. They include some special analyzers which monitor aquatic animal movements, as well as respirometers, enzymatic methods, and gas chromatographs. There is no uniform response of organisms to toxic substances. Also, a single all-purpose monitor to determine the state of human health in adverse environmental conditions does not exist. The best approach in such an evaluation would be to select a technology which at the least cost alerts the operator to the presence of most of the potential toxics in water, but once alerted, the operator must take a sample for a more specific analyses.

Only one analyzer was selected from each of the five categories of analyzers discussed and listed in Table 2 as a potential on-line analyzer.

All the equipment mentioned in the table requires additional research. Both the Microtox and respirometry show promise as early warning devices for the largest number of toxics. LDH and PID/FID, although promising approaches, still would require considerable research and are limited to organic halides. Gas chromatography is limited to detecting specific chemicals that can be used as surrogates, but for a specific application, this concept should be ignored.

TABLE 2

POTENTIAL ON-LINE ANALYZERS

Category	Analyzer	Comments
Life Function	Microtox	Available commercially but only for manual operation. Analyzer shows promise and is capable of detecting most toxic materials.
Enzymatic Response	Lactate dehydrogenase	Technique is still being researched. Analyzer would be sensitive to only organics halides.
Respirometer	Commercially available units	On-line equipment available but technique needs more research to assure necessary sensitivity. It may be necessary to develop specific microorganisms for use as a seed for water quality monitoring.
TOX	PID/FID on PID/ECD	Technique is simple but research is needed to define meaning of ratios. Field evaluation is also necessary.
Gas Chromatography	Commercially available units	Limited to specific compounds. Research needed to define surrogate compound and determine if application is of value.

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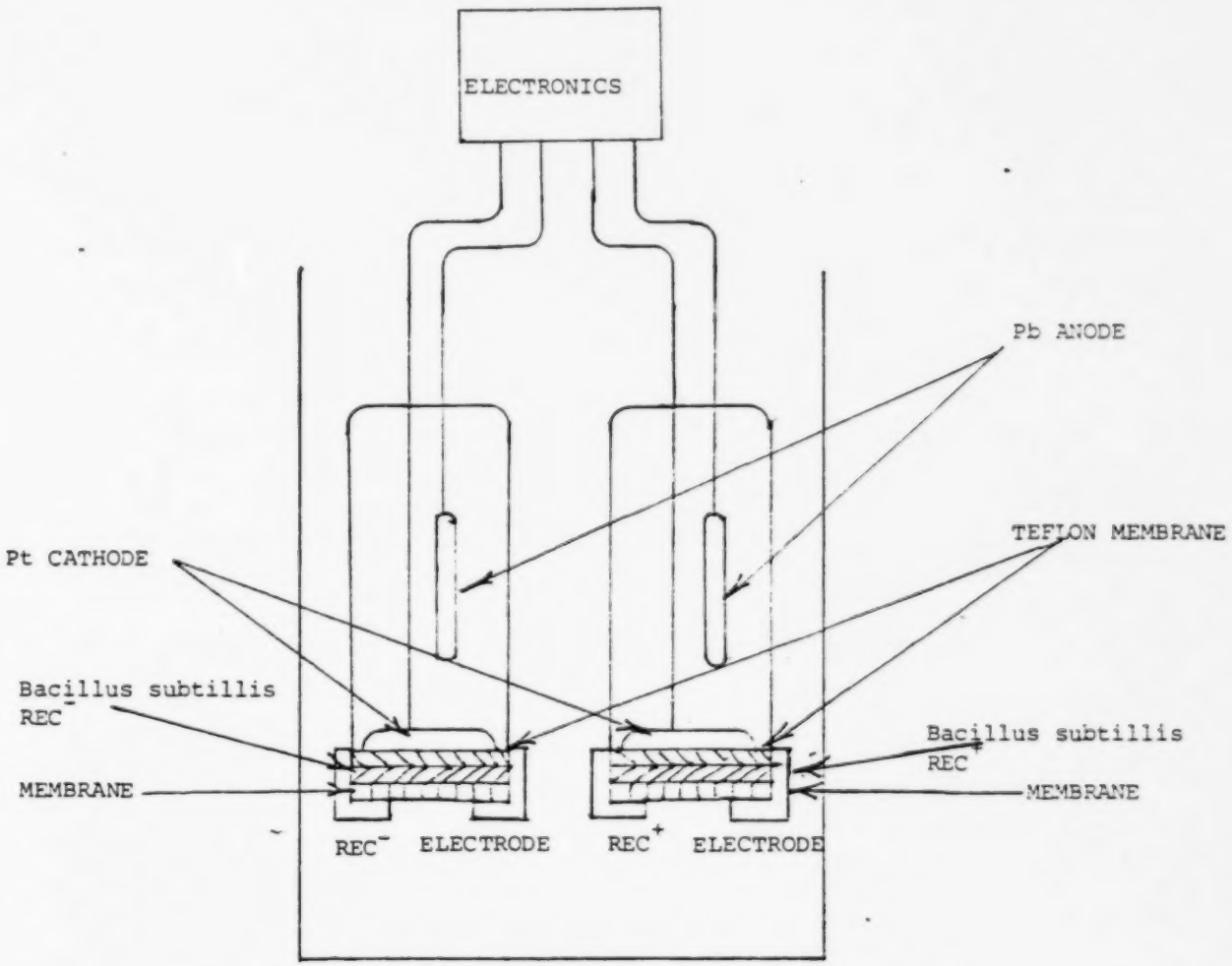


FIGURE 1

SCHEMATIC DIAGRAM OF MICROBIAL ELECTRODE

from Karube et.al. [13]

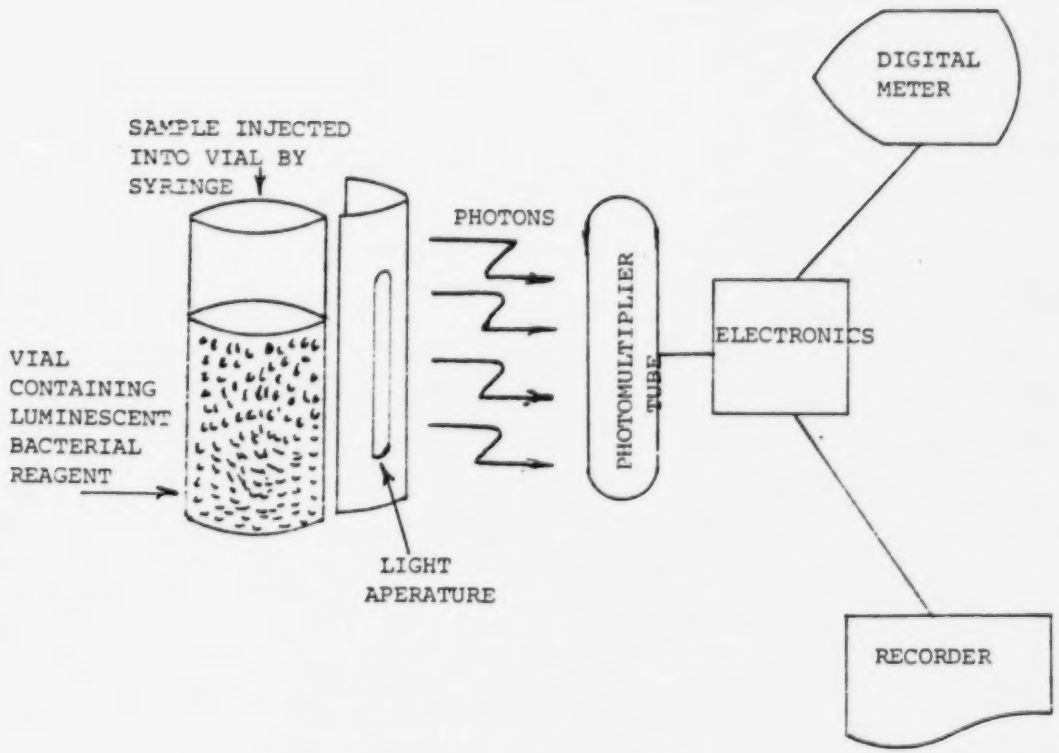
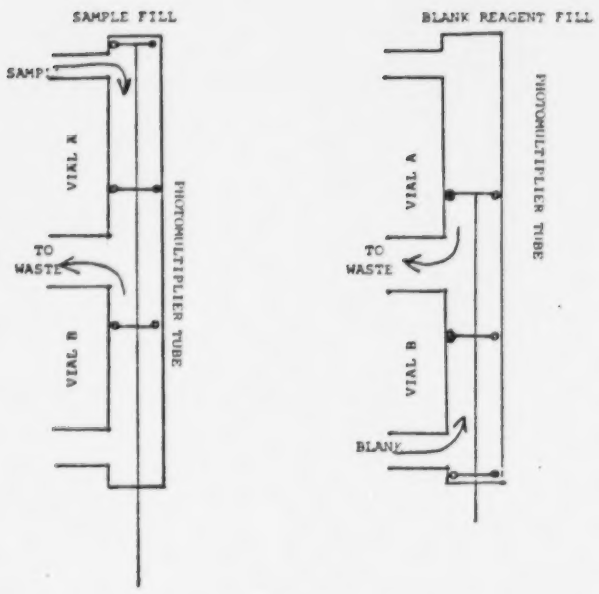


FIGURE 2

SCHEMATIC ILLUSTRATION OF MICROTOX



ACTION OF VIAL IN PROPOSED AUTOMATIC ANALYZER

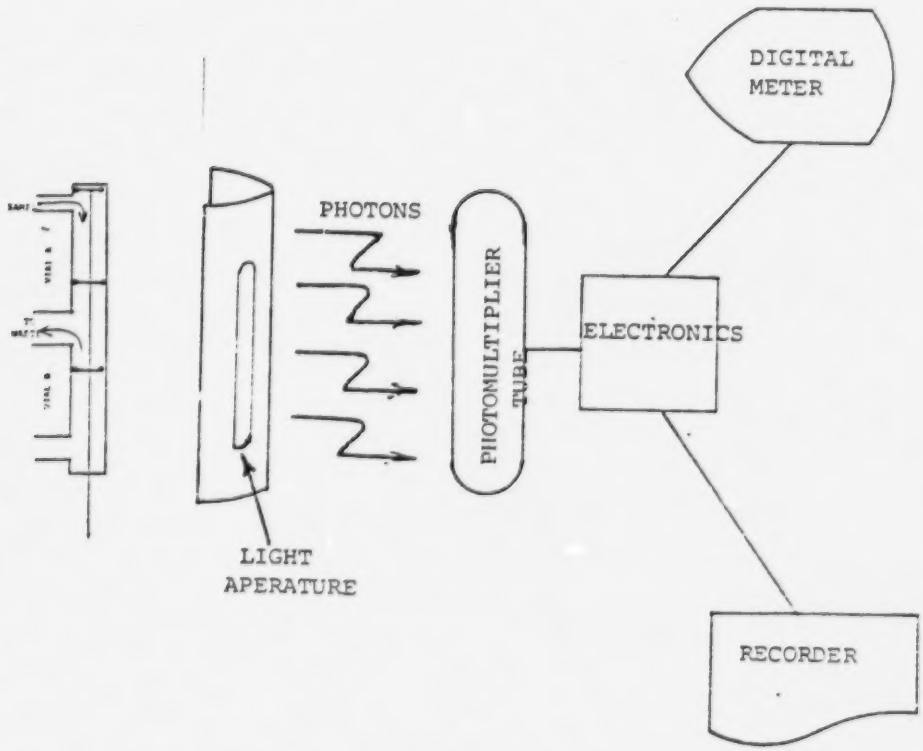


FIGURE 3
SCHEMATIC OF REVISIONS FOR MICROTOX FOR AUTOMATIC ANALYSIS